Subject: FR Notice Comments - 74FR14556 - Ocular Peer Panel Meeting
Date: Friday, May 15, 2009 2:42 PM

Below is the result of your feedback form. It was submitted by
() on Friday, May 15, 2009 at 14:42:58

Comment_date: May 15, 2009

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Comments: Where are the stratified human corneal epithelial cell models?

The following ATLA article provides a good overview of many of the ocular methods being reviewed this year by ICCVAM and ECVAM:
Eskes, et al. (2005). Eye irritation. Altern. Lab. Anim. [ATLA] 33, Suppl. 1, 47-81.

If you take the time to browse through this ATLA article, the one method you will notice that is missing from the 2009 Ocular Panel review is the Gillette HCE-T Model (page 57).

I'd like to take this opportunity to correct a number of

errors present in the 2005 ATLA article section that describes the HCE-T model (page 57), and to provide some references for those who would like to learn more about the performance of that method for ocular toxicity testing.

1) Key references were omitted from that review, and are provided below.

2) The prevalidation study involved testing only surfactant-containing formulations, however, the 4-lab validation study included both surfactant formulations and surfactants. A summary of the prevalidation study results was published along with a detailed description of the mechanistic basis of the test method and the biological relevance of the model (Ward, et al., 2003). The validation study results were written up in the form of a Background Review Document, but the company decided to not submit or publish the results.

3) Fields of application: The validation study focused on surfactant formulations and surfactants. A previous publication (Kruszewski, et al., 1997) provided the results of testing other chemical classes using this test method.

Prior to the conduct of the validation study, cationic surfactants were identified as incompatible with the fluorescein permeability (TEP) assay, due to the mechanism of action of that kind of surfactant on the cells. Cationics fix the cells in place, but the cells are dead and permeable and therefore take up the fluorescein. This prevents a quantitative leakage of fluorescein through the cell layers into the basal chamber. Other cytotoxicity assays are compatible with the HCE-T model (MTT, lactate, etc.), and can be used for testing cationic surfactants.

4) The HCE-T TEP method was useful for determining the ocular toxicity of substances across the range of in vivo ocular irritation, but may not have been sufficiently evaluated with severe materials which must be tested in diluted form when used in the 5 minute exposure protocol.

5) The method was extremely sensitive, and substances causing slight differences in degree of irritation could be reproducibly distinguished. A different assay

(transepithelial electrical resistance, TER) which evaluates disruption to the surface cell tight junctions was an even more sensitive indicator of ocular injury.

A battery of 3 endpoints was evaluated for a limited number of materials, and found to be even more predictive of the Draize score than the TEP data alone.

The ATLA article says that “histomorphology can also be used as an endpoint.” In my opinion, histomorphology was very useful in understanding the mechanism of action of chemicals on the cells; I would not use it as an endpoint.

6) On-going developments: None known, although the cells are available from the ATCC. ATCC reports for many years indicated that many companies and academic labs purchased and used the cells for research and internal testing applications.

The membrane/culture insert used during these studies may no longer be available. Data developed before this membrane was selected for the HCE-T model showed that the cells grew and stratified equally well on several other commercially-available inserts (and poorly on some).

7) INCORRECT last statement in ATLA article: The validation study was NOT restricted to surfactant-containing formulations. Both the prediction model and the test materials consisted of surfactants and surfactant formulations. A major reason for limiting the study to these types of materials was the difficulty in getting a sufficient number of other types of test materials with quality in vivo data for the study. The error in this last statement is surprising considering that 3 of the authors on this paper were associated with and had direct access to all of the validation study documents and data.

Summary:
Newer versions of stratified human corneal epithelial cell models have been developed. They probably share many or all of the same characteristics as the HCE-T model, so the data and experience from prior studies using this model should be useful in guiding new validation studies.

Key HCE-T References:

Ward, S.L. Gacula Jr., M., and Edelhauser, H.F. (2003). The Human Corneal Epithelial HCE-T TEP Assay for Eye Irritation: Scientific Relevance and Summary of Prevalidation Study Results. In: Alternative Toxicological Methods for the New Millennium. (Eds. H. Salem & S.A. Katz). CRC Press, Boca Raton, FL. pp. 161-186.

Clothier, R., Orme, A., Walker, T.L., Ward, S.L., Kruszewski, F.H., DiPasquale, L.C., and Broadhead, C.L. (2000). A comparison of three cytotoxicity assays using the corneal HCE-T model. Altern. Lab Anim. 28, 293-302.

Kruszewski, F.H., Walker, T.L. and DiPasquale, L.C. (1997). Evaluation of a human corneal epithelial cell line as an in vitro model for predicting ocular irritation. Fundam. Appl. Toxicol. 36:130-140.

Ward, S.L., Walker, T.L., and Dimitrijevich, S.D. (1997). Evaluation of chemically-induced toxicity using an in vitro model of human corneal epithelium. Toxicol. In Vitro. 11, 121-39.

Ward, S.L. (1996). Research needs for the development of improved alternatives to the Draize eye test.
In “Replacing the Draize Eye Irritation Test: Scientific Background and Research Needs” by the ILSI Health and Environmental Sciences Institute Technical Committee on Alternatives to Animal Testing. J. Toxicol. Cut. Ocul. Toxicol. 15, 224-29.

Kruszewski, F.H., Walker, T.L., Ward, S.L., and DiPasquale, L.C. (1995). Progress in the use of human ocular tissues for in vitro alternative methods. Comments on Toxicol. 5, 203-24.

Kahn, C.R., Young, E., Lee, I.H. & Rhim, J.S. (1993). Human corneal epithelial primary cultures and cell lines with extended life span: In vitro model for ocular studies. Invest. Ophthalmol. Vis. Sci. 34:3429-3441.

Documents submitted to NICEATM-ICCVAM:

Gillette 12/6/99 BRD (1999). Prevalidation / Validation Study for the HCE-T TEP assay (Pre-study plan).

Gillette 5/11/00 Report (2000). Responses to comments and

questions from the ICCVAM Ocular Toxicity Working Group, and modifications to the December 6, 1999 Background Review Document "Prevalidation / Validation Study for the HCE-T TEP Assay."

Gillette 1/29/01 BRD. (2001). Prevalidation Study Results for the HCE-T TEP Assay Background Review Document.

References for related human conjunctival epithelial cell model:

Smit, E.E., Sra, S.K., Grabowski, L.R., Ward, S.L., and Trocme, S.D. (2003). Modulation of IL-8 and RANTES release in human conjunctival epithelial cells: Primary cells and cell line compared and contrasted. Cornea 22, 332-337.

Ward, S., Walker, T., Trocme, S., Hallberg, C., Kruszewski, F., and DiPasquale, L. (2000). A human conjunctival model for the evaluation of eye irritants. In: Progress in the Reduction, Refinement and Replacement of Animal Experimentation. (Eds: M. Balls, A.-M. van Zeller, and M.E. Halder). Elsevier Science B.V., Amsterdam.
